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Effect of Bone Marrow–Derived Mononuclear Cell Treatment, Early or Late After Acute Myocardial Infarction

Twelve Months CMR and Long-Term Clinical Results

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Rationale: **Intracoronary delivery of autologous bone marrow–derived mononuclear cells (BM-MNC) may improve remodeling of the left ventricle (LV) after acute myocardial infarction (AMI).**

Objective **: To demonstrate long-term efficacy of BM-MNC treatment after AMI.**

Methods and Results **: In a multicenter study, we randomized 200 patients with large AMI in a 1:1:1 pattern into an open-labeled control and 2 BM-MNC treatment groups. In the BM-MNC groups, cells were either administered 5 to 7 days (early) or 3 to 4 weeks (late) after AMI. Cardiac magnetic resonance imaging was performed at baseline and after 12 months. The current analysis investigates the change from baseline to 12 months in global LV ejection fraction, LV volumes, scar size, and N-terminal pro-brain natriuretic peptide values comparing the 2 treatment groups with control in a linear regression model. Besides the complete case analysis, multiple imputation analysis was performed to address for missing data. Furthermore, the long-term clinical event rate was computed. The absolute change in LV ejection fraction from baseline to 12 months was −1.9±9.8% for control (mean±SD), −0.9±10.5% for the early treatment group, and −0.7±10.1% for the late treatment group. The difference between the groups was not significant, both for complete case analysis and multiple imputation analysis. A combined clinical end point occurred equally in all the groups. Overall, 1-year mortality was low (2.25%).**

Conclusions **: Among patients with AMI and LV dysfunction, treatment with BM-MNC either 5 to 7 days or 3 to 4 weeks after AMI did not improve LV function at 12 months, compared with control. The results are limited by an important drop out rate.**

Clinical Trial Registration Information**: URL: http://www.clinicaltrials.gov. Unique identifier: NCT00355186.**

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Key Words: bone marrow ■ cell transplantation ■ magnetic resonance imaging ■ myocardial infarction ■ stem cell

It has been suggested that progenitor cell–based therapy using autologous bone marrow might represent a source to improve left ventricular (LV) function in patients with acute myocardial infarction (AMI), when administered after successful percutaneous coronary intervention (PCI). Meta-analyses using publication-based data sets of the so far published randomized controlled clinical trials revealed mostly modest, but significant benefits on LV remodeling.[1–6] The largest benefit has attributed to patients of younger age and with a severely impaired LV ejection fraction (LVEF).[1] Most recently, the only meta-analysis using individual patient data[7] challenged these earlier results by showing no improvement of LVEF, ventricular remodeling, or clinical events with autologous bone marrow–derived mononuclear cells (BM-MNC). The individual results of the trials, which were included in that meta-analysis, were

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Nonstandard Abbreviations and Acronyms

AMI	acute myocardial infarction
BM-MNC	autologous bone marrow–derived mononuclear cells
CMR	cardiac magnetic resonance imaging
LVEDV	left ventricular end-diastolic volume
LVESV	LV end-systolic volume
NT-proBNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association
PCI	percutaneous coronary intervention
RCT	randomized controlled trial
STEMI	ST-segment–elevation myocardial infarction
TIME	Timing in Myocardial Infarction Evaluation

heterogeneous with respect to surrogate end points, such as LVEF or changes in LV volume. Moreover, their key outcomes had been mainly analyzed within a rather short follow-up period of 4 to 6 months after administration of BM-MNC.[8–17]

The SWISS-AMI study[18] assessed whether early (5–7 days) or late (3–4 weeks) intracoronary delivery of BM-MNC will affect the recovery of LV function after AMI. At 4 months follow-up, no significant change in LVEF in the treatment groups when compared with control was seen.[13] However, less is known about the long-term effects of BM-MNC therapy on LV remodeling. In the Repair-AMI trial, Assmus et al[19,20] reported a significant reduction of adverse clinical events and a sustained improvement of LV function after BM-MNC treatment at 2 and 5 years, respectively. Likewise, in line with an earlier meta-analysis,[4] the initially observed improvement in LVEF was maintained ≤5 years of follow-up in an early pilot study of our own group.[21] In contrast, in a randomized trial by Meyer et al,[22] LV function as assessed by cardiac magnetic resonance imaging (CMR) decreased by about 3 absolute points in both the control and BM-MNC–treated groups at 5 years.[22] Most recently, Traverse et al[23] reported the 1-year CMR results of the Timing in Myocardial Infarction Evaluation (TIME) trial and confirmed their earlier neutral results of BM-MNC therapy. Here, we report the completed 1-year CMR and biohumoral results of the SWISS-AMI trial and the available clinical event rates ≤5 years of follow-up.

Methods

Study Sample and Protocol

The entire study design with predefined inclusion and exclusion criteria have been previously described.[13,18] In brief, patients with acute ST-segment–elevation myocardial infarction (STEMI) and successful PCI within 24 hours after symptom onset were eligible for enrollment into this multicenter, randomized, controlled trial (RCT) provided they presented with an LVEF of <45% as assessed by an LV angiogram or transthoracic echocardiography the day of or after the AMI. After giving their informed consent to participate to the study, patients were randomly assigned in a 1:1:1 fashion to 1 open-labeled control and 2 BM-MNC treatment groups. The control group received best medical management according to current guidelines,[24] including aspirin and clopidogrel or prasugrel, statins, β-blockers, and ACE-inhibitors or ATII-receptor blockers, as well as aldosterone antagonists, if indicated. The early treatment group received intracoronary BM-MNC infusion 5 to 7 days and the late treatment group 3 to 4 weeks after primary PCI, on top of the best medical management. LV function was assessed by CMR in all patients at baseline, at 4 and 12 months after AMI. The hypothesis was that both the treatment groups had equal efficacy to raise LVEF when compared with control patients. A total of 4 Swiss tertiary centers (University Hospital, Zurich; Bern University Hospital; Cantonal Hospital; and Lucerne and Fondazione Cardiocentro Ticino) participated in this trial. The study was conducted in accordance with the declaration of Helsinki, and the protocol was approved by the regional ethical Committee of each participating center and by the Federal competent authorities (Swissmedic and Federal Office of Public Health).

Bone Marrow Aspiration, Cell Processing, and Intracoronary Infusion

All procedures have been precisely described elsewhere.[13,18] In brief, bone marrow samples were collected under sterile conditions from the iliac crest in local anesthesia. The cell processing was entirely performed in a centralized, good manufacturing practice–certified facility (Cell Therapy Unit, Cardiocentro Ticino, Lugano, CH, Switzerland) by using density gradient centrifugation. The mononuclear cell fraction was then resuspended in 10 mL of serum-free medium added with 20% of autologous serum without adding any heparin.

Cell viability, functional testing, characterization of the product, and sterility were assessed from an aliquot of the cell suspension. BM-MNC were then administered in the former infarct-related vessel by performing the previously described stop-flow technique.[13,18]

CMR Imaging

As previously described, cardiac imaging was performed using 1.5-Tesla clinical magnetic resonance systems with dedicated cardiac phased–array receiver coils for signal reception. All patients underwent CMR studies during the hospitalization for the AMI (baseline). The studies were then repeated after 4 and 12 months. Functional imaging of the LV was performed by means of standard ECG-triggered steady state–free precession acquisitions during repetitive breath holds in 3 long-axis orientations and in contiguous short-axis orientation, covering the entire LV. A bolus of a conventional extracellular gadolinium-chelates contrast medium at a dose of 0.20 mmol/kg of body weight was administered to assess myocardial scar imaging, by using an inversion recovery fast gradient echo imaging sequence.[25–28] Scar imaging was performed 20 minutes after administration of contrast medium in identical locations as functional data were acquired.

CMR data analysis was performed in a core laboratory (University Hospital Zurich/CH, Switzerland) using dedicated cardiac analysis software (GTVolume, Gyrotools Ltd, Zurich/CH). LV end-diastolic (LVEDV) and end-systolic (LVESV) volumes, LVEF, and LV mass have been quantified for the assessment of the change of LVEF and for the assessment of ventricular remodeling over time in the 3 study groups. Scar mass was assessed in grams (g).

Clinical Follow-Up

Adverse clinical events, Canadian Cardiac Society (CCS), and New York Heart Association (NYHA) class were assessed at 4 and 12 months after AMI performing dedicated follow-up visits, which were scheduled together with the CMR data acquisition. Thereafter, systematic annual telephone visits were performed ≤5 years after AMI. During these visits, clinical events were systematically asked directly to the patient by trained medical study investigators. Patients were classified as alive only if a direct contact could have been established.

N-Terminal Pro-Brain Natriuretic Peptide

As per protocol, N-terminal pro-brain natriuretic peptide (NT-proBNP) values were supposed to be assessed for the entire study population at baseline, during 4 and 12 months follow-up, and the analysis was performed in a central laboratory (Department of Clinical Chemistry, University Hospital, Zurich, Switzerland).

End Points

The following end points are presented: change in LV function and remodeling at 12 months follow-up (LVEF, LVEDV, LVESV, and infarct size) as well as the change of NT-proBNP over the entire follow-up. We also report on the mid- to long-term clinical follow-up and events

(survival and major adverse event–free survival ≤5 years). The following major adverse events are included in the combined end point: all-cause death, recurrence of myocardial infarction, any coronary revascularization procedure, stroke, or rehospitalization for heart failure.

Statistical Analysis

Baseline Characteristics

Continuous data are described either as mean and SD or as median and 25th to 75th percentiles, whenever more appropriate. Categorical data are expressed as counts and percent. Comparison between treatment arms at baseline is performed with the Kruskal–Wallis test and the Fisher exact test, respectively. Adherence to state-of-the-art pharmacological treatment after STEMI and depressed LV function was assessed at baseline and after 4 and 12 months. Only descriptive statistics of the difference between groups are shown.

Evaluation of Follow-Up Data Through 1 Year

To assess whether profiles over time are different between the 3 treatment arms, comparisons of the changes over time (ie, between baseline, 4 months, and 12 months) of the above-described end points (including NT-proBNP values) between the 3 treatment groups have been performed. To do so, a multivariable linear regression model for repeated measures has been fitted, including the factors treatment and time as well as their interaction. Huber White robust SEs were computed to account for intrapatient correlation of measures over time. Given that 5 different end points are assessed, interaction is considered statistically significant when the P value is <0.01 (Bonferroni correction). For all other exploratory (unadjusted) post hoc comparisons, P values are to be considered descriptive only. In all models, treatment effect was adjusted for age, sex, history of coronary artery disease, diabetes mellitus, and baseline LVEF. Residuals were graphically inspected to assess model fit.

Median follow-up was described using the inverse Kaplan–Meier method. Cumulative (event-free) survival was plotted using the Kaplan–Meier method and compared with the log-rank test. Time to the first event was considered. The primary comparisons were performed between control and the 2 treatments arms (early and late). Further comparisons of control and 1 combined treatment arm (early+late) are shown in the Online Data Supplement.

Adjustment for Missing Data

The drop out rate, especially for the CMR protocol and within the late therapy group, was high. To account for missing data, we therefore performed multiple imputation analysis using chained equation,[29] which fills in missing values in multiple variables iteratively. Fifty data sets were generated after setting a seed for reproducibility. The largest fraction of missing information was used to confirm that the number of imputed data sets was adequate (thumb rule M=100×fraction of missing information). We imputed the LVEF, LVEDV, LVESV, scar size, NT-proBNP, and their changes through 1 year. We used the following independent variables for imputation: age, body mass index, sex, hypertension, dislipidemia, diabetes mellitus, smoking (active/previous), family history of coronary artery disease, 1-, 2-, or 3-vessel disease, treatment arm, time from pain to revascularization, TIMI flow before PCI, TIMI flow after PCI, use of glycoprotein IIb/IIIa inhibitors, maximal creatinine kinase values, heart failure at baseline, and ventricular fibrillation at presentation. The imputed data were then described with the mean and SE or the median and SE obtained via quantile regression. The same regression models as in the complete case analysis were fitted (while accounting for imputation). Details and discussion of the reasons for missingness are given in the Online Data Supplement.

All tests were 2 sided. The analysis followed the intention-to-treat principle. Stata 14 and Stata 14 mi suite (StataCorp, College Station, TX) were used for computation.

Results

A total of 200 patients were enrolled in the study by 4 centers from October 2006 to January 2012. Sixty-six of them were randomized in the early BM-MNC treatment group and 67 each in the control and late treatment groups, respectively. Complete paired CMR analysis (both at baseline and at 12 months) was available for 150 patients (75%). Twenty-eight patients were lost for follow-up because of death (n=4), implantable cardioverter–defibrillator implantation between 4 and 12 months (n=5), from the CMR protocol because

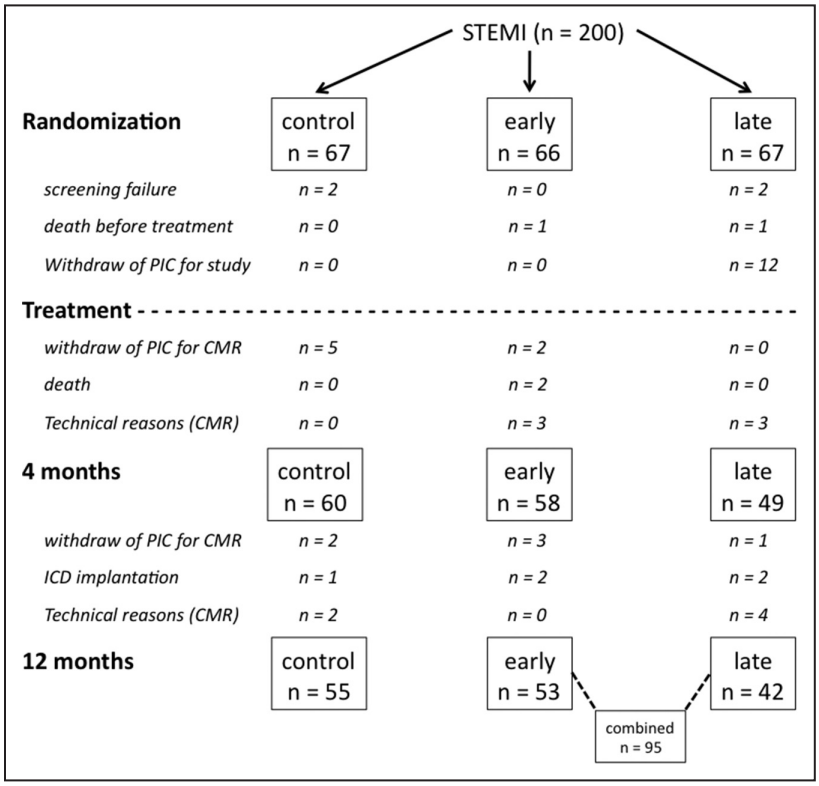

Figure 1. Flow diagram of patient enrollment until 12-mo follow-up. CMR indicates cardiac magnetic resonance imaging; ICD, implantable cardioverter–defibrillator; PIC, patient informed consent; and STEMI, ST-segment–elevation myocardial infarction.

of patient's preference (n=7), and because of technical reasons (n=12). For 178 patients (89%), clinical follow-up was available at 12 months (22 patients withdrew their informed consent to participate in the study). NT-proBNP values were assessed in 175 patients at baseline, in 169 patients at 4 months of follow-up, and in 163 patients at 12 months of follow-up. Complete patient flow from randomization to 12 months of follow-up is shown in Figure 1.

Baseline Characteristics

The patient's baseline characteristics have been previously published[13] and are shown in Online Table I. Briefly, patients in the late treatment group were older, were less frequently smokers, and had a higher rate of bivalirudin use during primary PCI. Furthermore, a trend toward a higher baseline LVEF in the control group was observed, which could never be entirely explained. Apart from this, there was no statistically significant difference in baseline characteristics between the 3 groups.

Most of the patients (92.3%) had anterior STEMI because of occlusion of the left anterior descending coronary artery, and the median time from onset of chest pain to reperfusion therapy was 4.5 hours (range: 2.75–8 hours). Antithrombotic treatment during PCI consisted of 65% of glycoprotein IIb/IIIa inhibitors and 11% of bivalirudin. The median of maximal creatine kinase plasma levels was 3919 U/L (range: 2203–5867), and baseline LVEF, as assessed by CMR at a median of 6 (range: 4–8) days after the index STEMI, was 37.4% (range: 30.9–44.1).

Pharmacological treatment (Table 1) was initiated according to current guidelines[24] shortly after primary PCI. Compliance to prescribed treatment was overall between 90% and 100% for dual-antiplatelet therapy, statins, β-blockers, ACE-inhibitors, or ATII-receptor blockers, respectively.

The characteristics of the infused BM-MNC have been described previously[13] and are shown in Online Table II. Briefly, a median of 153×10^6 nucleated cells have been infused in the infarct-related artery at a median of 6 days (range: 5–7) after STEMI in the early and after a median of 24 days (range: 21–28) after the event in the late treatment group.

Evaluation of Follow-Up Data Through 1 Year

The change in mean LVEF over time within the different groups is shown in Table 2 (Complete Case Analysis); Figure 2 and the absolute change from baseline to 12 months is shown in Figure 3. Specifically, in exploratory comparisons, for both the therapy groups, between baseline and 4 months follow-up, a nonsignificant trend toward an increase in LVEF with a subsequent decrease between 4 and 12 months was observed. In the control group, a constant, but nonsignificant, decrease in LVEF was noticed. The absolute within-group differences from baseline to 12 months were −1.9 (95% confidence interval, −4.5 to −0.8) for control, −0.9 (−3.8 to 2.0) for the early, and −0.7 (−3.9 to 2.4) for the late therapy groups (Table 2 [Complete Case Analysis]).

Between baseline and 4 months, negative remodeling of the LV occurred predominantly in the control and early treatment groups, whereas the late therapy group showed a nonsignificant increase of both LVEDV and LVESV (Table 2 [Complete Case Analysis]). In all patient groups, LV volumes did not further increase from 4 to 12 months, indicating that the remodeling process had been mainly concluded at 4 months. The absolute within-group differences from baseline to 12 months for LVEDV and LVESV were 19 mL (range: 5–33) and 17 mL (range: 6–29) for the control group, 26 mL (range: 13–38) and 21 mL (range: 10–32) for the early treatment group, and 11 mL (range: −1 to 23) and 10 mL (range: −0 to 21) for the late therapy group, respectively. As expected, in all groups, total scar mass uniformly and significantly decreased between baseline and 12 months without any difference between groups (Table 2 [Complete Case Analysis]). At multivariable repeated measures analysis, none of LVEF, LVEDV, LVESV, and scar size showed differences over time between control and the 2 treatment arms, as documented by the nonsignificant interactions (Table 2 [Complete Case Analysi)]; Online Table III for model details).

Additional comparisons between control and the combined treatment arm (early+late) are shown in the Online Data Supplement (Online Figure I). The results were not different from the initial comparisons between the 3 distinct groups.

As expected, NT-proBNP values were highest at the time of the event and then decreased significantly in all the 3 groups ≤4 months of follow-up. In terms of absolute change between baseline and 12 months of follow-up, there was a trend toward a more pronounced improvement of NT-proBNP values in favor of both the therapy groups (Figure 3). However, with multivariable repeated-measures analysis, no difference was seen any longer between the 3 groups (nonsignificant interactions in Table 2 [Complete Case Analysis]; Online Table IIIA for model details).

Table 1. Descriptive Statistic of the Pharmacological Treatment of the Included Patients at Baseline, After 4 and 12 Months

	Control (n=67)	Early (n=65)	Late (n=63)
Aspirin	98.5/98.4/95.7	98.4/98.4/95.7	98.3/98.2/97.4
P2Y12 antagonist	100/100/93.5	100/100/86.7	100/98.2/92.3
ACE-inhibitor or ATII-receptor blocker	95.5/100/100	100/100/93.5	96.6/98.2/97.5
β-Blocker	86.4/85.2/87.0	91.9/88.5/84.8	93.2/92.6/89.7
Aldosterone antagonist	12.1/11.5/8.7	12.9/14.8/10.9	15.2/9.3/15.4
Statin	97.0/98.4/95.7	100/95.1/93.5	98.3/100/100

Medication at discharge/after 4/and 12 months, %. ACE indicates angiotensin-converting enzyme; and AT, angiotensin.

Table 2. Comparison of Changes Over Time in the 3 Study Arms

	Complete Case Analysis				Multiple Imputation			
	Control (n=55)	Early (n=53)	Late (n=42)	*P* Value for Interaction*	Control (n=55)	Early (n=53)	Late (n=42)	*P* Value for Interaction*
LVEF, %								
Baseline	40.0 (9.9)	36.5 (9.9)	36.3 (8.2)	0.640	39.9 (1.3)	35.7 (1.3)	36.3 (1.1)	0.688
4 mo	39.6 (12.0)	37.9 (10.3)	37.4 (9.7)		39.6 (1.5)	37.2 (1.4)	37.0 (1.4)	
12 mo	38.1 (13.6)	36.2 (11.4)	36.6 (12.2)		38.6 (1.7)	35.2 (1.5)	36.7 (1.7)	
Δ bl–12 mo	–1.9 (–4.5 to –0.8)	–0.9 (–3.8 to 2.0)	–0.7 (–3.9 to 2.4)		–1.8 (–4.4 to 0.7)	–0.9 (–3.7 to 1.9)	–0.6 (–3.4 to 2.2)	
LVEDV, mL								
Baseline	153 (38)	156 (41)	157 (37)	0.063	153 (5)	158 (6)	157 (5)	0.292
4 mo	180 (52)	183 (55)	167 (45)		178 (7)	182 (7)	167 (7)	
12 mo	170 (56)	179 (61)	164 (47)		170 (7)	178 (8)	163 (7)	
Δ bl–12 mo	19 (5 to 33)	26 (13 to 38)	11 (–1 to 23)		20.5 (6.6 to 34.3)	26.0 (14.0 to 38.1)	10.3 (–2.0 to 22.6)	
LVESV, mL								
Baseline	94 (33)	100 (36)	100 (29)	0.199	94 (4)	103 (5)	100 (4)	0.490
4 mo	112 (46)	117 (51)	107 (40)		111 (6)	117 (7)	107 (6)	
12 mo	110 (53)	118 (56)	107 (44)		109 (7)	119 (7)	107 (7)	
Δ bl–12 mo	17 (6 to 29)	21 (10 to 32)	10 (–0 to 21)		18.6 (6.8 to 30.3)	22.0 (11.4 to 32.5)	10.0 (–0.4 to 20.5)	
Scar size, g								
Baseline	45.3 (28.0)	44.0 (22.3)	38.5 (22.5)	0.181	44.0 (3.4)	45.4 (3.0)	38.0 (3.0)	0.702
4 mo	29.2 (15.7)	28.9 (15.7)	24.3 (11.1)		29.2 (2.2)	30.3 (2.2)	25.5 (2.2)	
12 mo	22.0 (12.2)	24.1 (13.1)	22.4 (13.1)		23.1 (2.2)	26.5 (2.2)	23.3 (2.5)	
Δ bl–12 mo	–23 (–29 to –16)	–22 (–28 to –17)	–16 (–24 to –8)		–21.4 (–27.9 to –14.9)	–23.5 (–29.5 to –17.6)	–16.6 (–23.7 to –9.4)	
NT-proBNP, pg/mL‡								
Baseline	1103 (737–2580)	1450 (901–2355)	1581 (937–2889)	0.404	1139 (227)	1508 (197)	1539 (244)	0.736
4 mo	416 (206–842)	501 (240–912)	467 (284–949)		440 (90)	534 (101)	557 (135)	
12 mo	332 (110–672)	291 (172–566)	330 (122–591)		360 (84)	310 (60)	362 (86)	
Δ bl–12 mo	–1012 (–1376 to –65)	–1624 (–2145 to –1103)	–1488 (–1828 to –1147)		–1048 (–1442 to –654)	–1681 (–2187 to –1175)	–1538 (–1936 to –1139)	

Complete Case Analysis: All values are given as mean (SD) or ‡median (25th–75th percentiles). Δ bl-12 months is change from baseline to 12 months and is given as mean (95% confidence interval). The number of the subjects in each population can be found in Figure 2. Multiple Imputation: All values are given as mean (SE) of the imputed variables or ‡median (SE). Δ bl-12 months is change from baseline to 12 months and is given as mean (95% confidence interval). The model has been fitted accounting for the imputation process. The model is adjusted for age, sex, history of coronary artery disease, diabetes mellitus, and baseline LVEF. The test for interaction tests whether the profile over time of the measured variables is different between treatment arms. *P* value for significance is set at 0.01 (Bonferroni correction). Details of the regression models results are given in the Online Table III. Bl indicates baseline; LVEDV, left ventricular end-diastolic volume; LVEF, LV ejection fraction; LVESV, LV end-systolic volume; and NT-proBNP, N-terminal pro-brain natriuretic peptide.

**P* value for interaction: derives from a general linear model for repeated measures, including both the main effects for treatment and time and their interaction.

Adjustment for Missing Data

The results of the comparisons of the different treatment groups after multiple imputation to account for missing data are shown in Table 2 [Multiple Imputation]. They seemed similar to the complete case analysis, both in the data description and the comparison of time profile, as can be easily identified by direct comparison with the results of the complete case analysis (Table 2 [Complete Case Analysis and Multiple Imputation]). Details of the regression models are reported in the Online Table IIIA and IIIB.

Clinical Follow-Up and Events

At 12 months, 84% of the patients were in NYHA class I and 98% of them were free of any angina (without any difference between groups). As shown in Table 3, the clinical event rate at 12 months was similar within the 3 groups for any of the

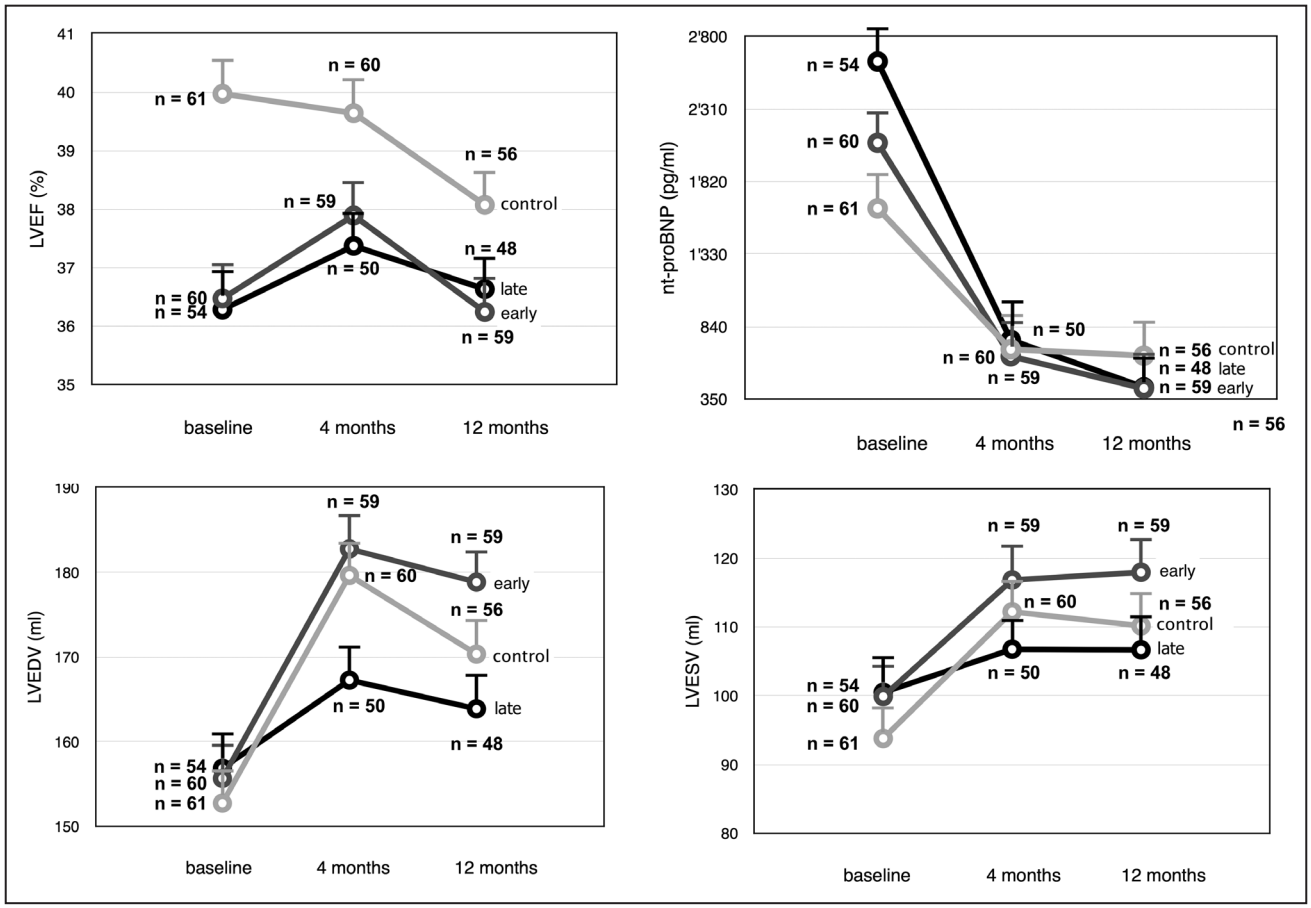

Figure 2. Descriptive statistic of the mean left ventricular ejection fraction (LVEF, %) values at baseline, after 4 and 12 months for control, early and late bone marrow–derived mononuclear cell treatment. LVEDV indicates LV end-diastolic volume; LVESV, LV end-systolic volume; and NT-proBNP, N-terminal pro-brain natriuretic peptide.

assessed events or their combination. Events occurring between randomization and cell therapy and during the cumulative 12 months thereafter are presented separately.

Clinical events were furthermore followed ≤5 years (median follow-up: 38 months). As shown in Figure 4, mortality remained low, that is, 2.25% at 12 months and 2.3% at 24 months, in all the 3 groups ≤5 years after the event without between-group differences (log-rank test P=0.43), regardless of the fact that high-risk patients with large STEMIs were enrolled. No death occurred in the control group, whereas 3 patients (5.8%) died in the early and 1 patient (2.3%) died in the late treatment groups. Of note, however, 2 deaths (1 in each treatment group) occurred between randomization and the scheduled BM-MNC treatment. Also, the major adverse event–free survival was comparable between the 3 groups (Figure 4, log-rank test P=0.65) for the combined end point of all-cause death, recurrent myocardial infarction, any coronary revascularization, and stroke or rehospitalization for heart failure.

Discussion

The Swiss-AMI trial is the largest RCT, which aimed to address LV function by CMR not only at 4 months after STEMI but also at 12 months of follow-up. Furthermore, together with the TIME trial,[16,23] it is the only RCT, which addressed 2 time points, either an early or a late time points after BM-MNC administration. Both the treatment groups were compared with a control arm in a unique trial design, assuming an equal treatment effect. Similar to what we reported at short term,[13] we were also unable to demonstrate a significant treatment effect of BM-MNC 12 months after the initial event in both the treatment groups. Although at 4 months a small increase in LVEF was observed in both the treatment groups, there was a nonsignificant decrease in LVEF from baseline to 12 months for all groups without any within-group difference. In particular, between 4 and 12 months, a trend toward a decrease in LVEF was apparent in all the 3 groups. Negative remodeling was not notable in the late treatment group, but was notable in the early treatment and control groups. The latter finding, however, is difficult to interpret because it was not associated with an improvement in LV function. Although the CMR protocol of the Swiss-AMI trial had a relatively high drop out rate, the robustness of the results was confirmed by the fact that multiple imputation analyses to address for missing data only marginally changed the outcomes of the study.

More than a decade after the publication of the first RCTs, the results of our study underscore that it is still unclear if BM-MNC, administered within the first weeks after STEMI, exert a beneficial effect on LV function during follow-up. The results

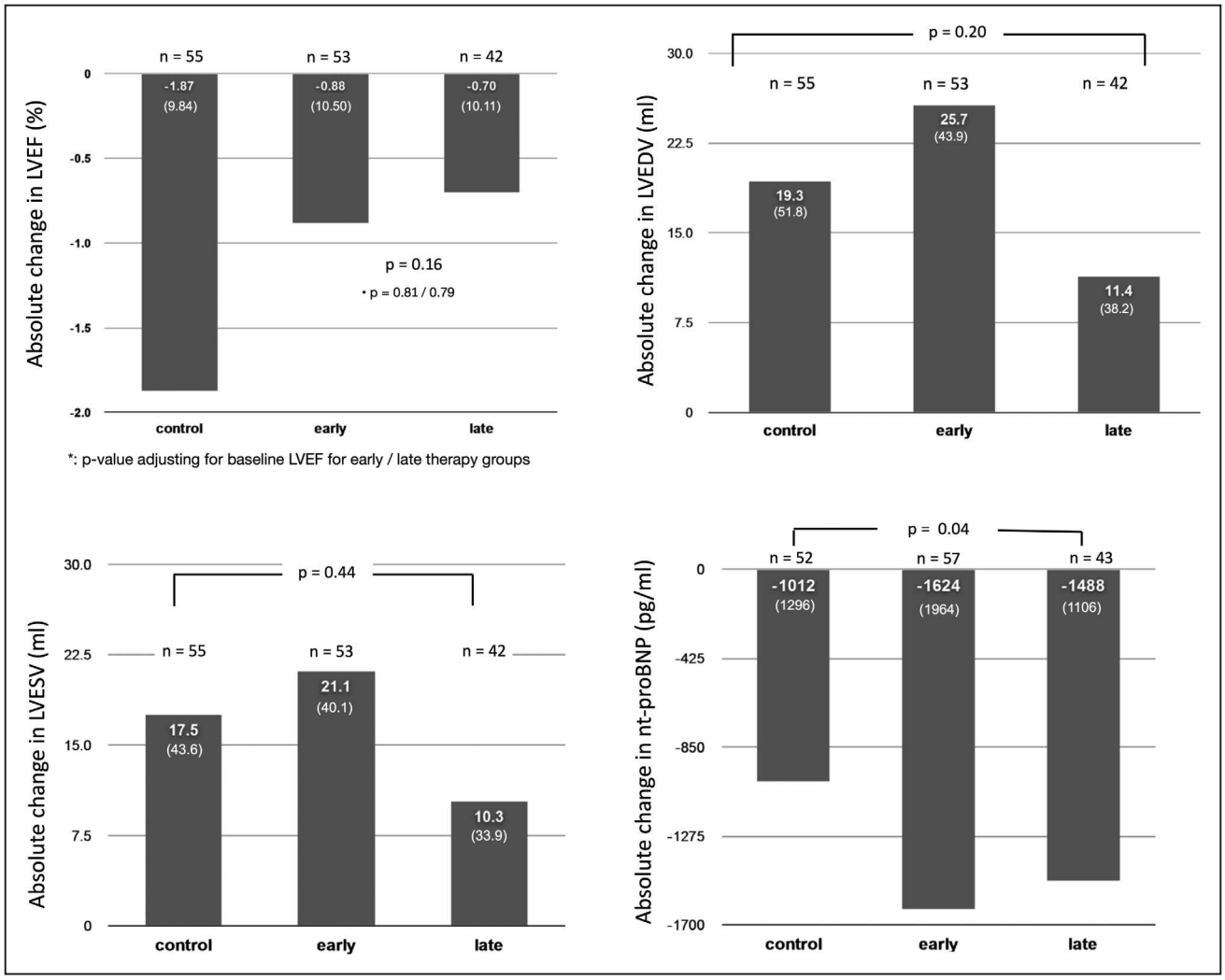

Figure 3. Descriptive statistic (columns) of the mean absolute change in left ventricular ejection fraction (LVEF, %), LV end-diastolic volume (LVEDV, mL), LV end-systolic volume (LVESV, mL), and N-terminal brain natriuretic peptide (pg/mL) from baseline to 12 mo for control, early and late bone marrow–derived mononuclear cell treatment.

of earlier RCTs[9,12,17] and those of several meta-analyses[1,2,5,6] were mainly suggesting a positive effect of BM-MNC treatment on LV function. Indeed, the earlier studies showed a small increase in LVE and a reduction in LV volumes and suggested potential clinical benefit. In contrast, more recent studies,[13,15,16] using CMR as a standard to assess LV function, were not able to confirm a significant effect of intracoronary BM-MNC administration in patients with STEMI undergoing successful primary PCI. Similarly, a recent meta-analysis, using individual patient data of the included trials,[7] did not show any effect on LVEF and was not able to confirm a positive effect on LV remodeling either. As a consequence, there is an ongoing debate how such discrepancies may be explained. Most of the meta-analyses were using publication-based data sets such as the number of patients in the treated and control groups and the mean changes from baseline to follow-up including the SD of both groups. An advantage of the meta-analysis based on individual patient data, such as the Meta-Analysis of Cell-Based Cardiac Studies (ACCRUE) consortium,[7] is the use of effectively collected data of each participating study and a lower heterogeneity between the included trials and the exclusion of withdrawn studies (such as the Strauer series) and those without published changes in LVEF (often the case in smaller studies). Such an approach avoids the necessity to recalculate changes based on mathematical formula. However, some trials had to be left out from the meta-analysis because of the unwillingness of the authors to provide individual patient data, which somewhat weakens the generalizability of that recent meta-analysis. Thus, also meta-analyses of any nature are not able to give the definitive answer to the still unresolved issue if the intracoronary injection of BM-MNC shortly after STEMI will improve LV function or outcome.

The durability of a putative beneficial effect of BM-MNC is even less well studied. The mid-term outcome of the Swiss-AMI trial between 4 and 12 months is partially in line with the 5-year results of Meyer et al[22] who also described a late decrease in LVEF of 3 absolute percentage points in the control and treatment groups. Furthermore, there are important similarities between our study and the TIME trial[16,23] with regard to the study design by addressing 2 different time points of cell administration and the use of CMR to assess LV function. Likewise, the results of both the trials and the conclusions seem

Table 3. Major Adverse Cardiac Events at 12 Months

	Control	Early	Late	*P* Value
Events between randomization and therapy				
Death	0	1 (1.9%)	1(2.3%)	0.24
Events at 12-mo follow-up (cumulative)				
Death	0	3 (5.8%)	1 (2.3%)	0.32
Myocardial infarction	2 (4.4%)	1 (2.0%)	0	0.25
Rehospitalization for heart failure	3 (6.5%)	1 (2.0%)	3 (7.1%)	0.50
Revascularization	6 (12.8%)	4 (7.8%)	4 (9.3%)	0.73
Cerebral infarction/TIA	2 (4.4%)	2 (4.1%)	0	0.55
ICD implantation	1 (2.2%)	2 (4.1%)	3 (7.1%)	0.48
Combined events				
Death, myocardial infarction, revascularization, and rehospitalization for heart failure	9 (19.3%)	8 (15.6%)	8 (18.6%)	0.88
Death, myocardial infarction, revascularization, and rehospitalization for heart failure or stroke	11 (23.7%)	10 (19.7%)	8 (18.6%)	0.77

ICD indicates implantable cardioverter–defibrillator; and TIA, transient ischemic attack.

strikingly similar, for both the short- and the long-term results. As in the TIME trial,[23] we also pooled both therapy groups in a secondary analysis without any substantial change of results.

The long-term clinical outcome of the Swiss-AMI trial was characterized by a surprisingly low overall mortality rate of only 2.25% at 1 year and of 2.3% at 2 years. Furthermore, no difference between the 3 groups was observed as for the combined clinical end point of death, myocardial infarction, revascularization of any kind, or rehospitalization for heart failure. The low mortality rate of our study at 1 and 2 years of follow-up leads to 2 further conclusions: first, mortality after large STEMIs in the era of modern reperfusion therapy with primary PCI presenting in a hemodynamically stable condition is much lower than generally expected. This could play a crucial role for the ongoing The Effect of Intracoronary Reinfusion of BM-MNC on All Cause-Mortality in AMI (BAMI) trial (NCT 01569178), which assumed a 12% of mortality rate after 2 years using similar inclusion criteria as the here presented SWISS-AMI trial. Second, as for all-cause mortality, also for the combined clinical end point, no significant difference could be detected between the control and treatment groups ≤38 months. Importantly, our results contradict the positive long-term results of the Repair-AMI study, to which our group participated as recruiting center, and the findings of several meta-analyses,[2,19] which were both showing a potential positive effect of BM-MNC treatment on mortality and outcome.

Interestingly, NT-proBNP, a biomarker of LV dysfunction, which is closely related to prognosis,[30,31] was the only parameter pointing to a potential treatment effect of BM-MNC. Only in both the BM-MNC therapy groups, a near normalization NT-proBNP levels occurred between 4 and 12 months, whereas in the control group, the values did not further decrease within this time period. The difference between groups tended to be significant

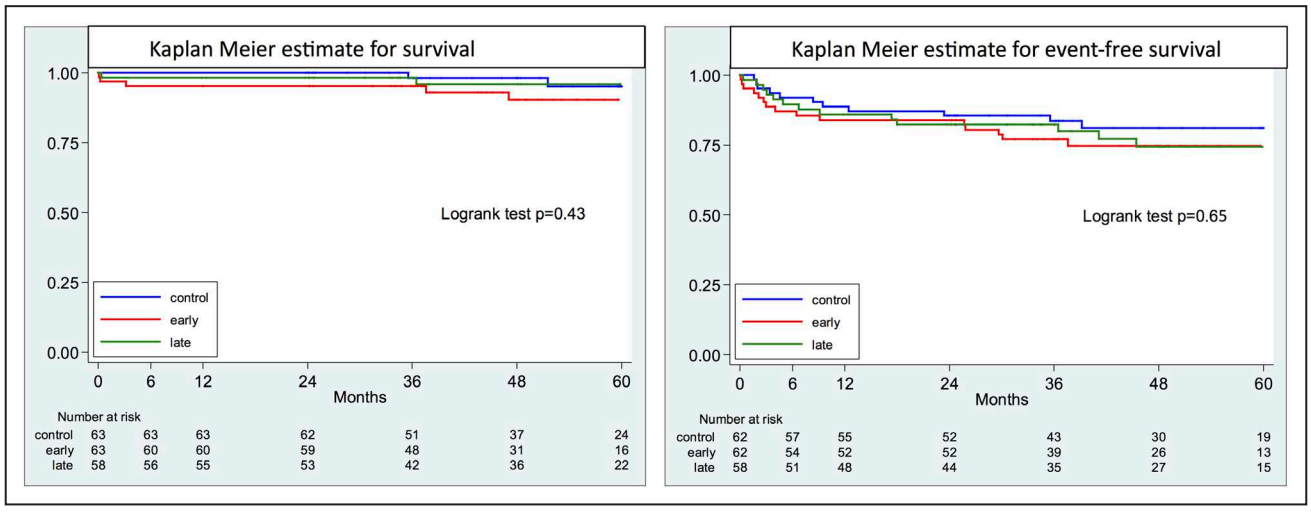

Figure 4. Kaplan–Meier curves for the incidence of death or the combined clinical end point of death, myocardial infarction, any coronary revascularization, rehospitalization for heart failure or stroke for control (blue curve), and early (red curve) or late bone marrow–derived mononuclear cell treatment (green curve).

only for absolute changes between baseline and 12 months, but not in the regression model and after accounting for missing data. Obviously, the role of NT-proBNP has never been validated as a marker of outcome for cell-based treatment studies, which limits the value of these data. Further investigations are, thus, required to clarify if NT-proBNP may be a helpful surrogate marker in addition to LVEF to assess the clinical use of cell-based therapy.

Study Limitations

The limitations of the SWISS-AMI trial have been extensively discussed previously.[13] One limitation is the important drop out rate. The withdrawal rate from the study protocol was higher in the late therapy group, particularly during the first 3 weeks, that is, during the waiting time between discharge from hospital after primary PCI and the planned rehospitalization for cell therapy. Likewise, the drop out rate of the CMR protocol was also high, but it was well comparable to those reported in other important trials of the field,[23] indicating that in general the designs of current trial protocols are challenging.

A further limitation of the study is the long recruitment period. Finally, the biological properties of the injected BM-MNC may have limited the treatment effect. BM-MNC are a heterogenic, unselected cell population, containing only small portions of cell types, which in vitro or in small-animal trials have been shown to exert regenerative capacity. Cell characterization at the time of the study enrollment was less well studied and was limited to the number of $CD34^+$ and $CD133^+$ cells (which were found in comparable numbers as in previous trials[13]). Nevertheless, the lack of regenerative capacity of BM-MNC in our trial cannot be generalized to the other selected cell types deriving from the bone marrow or for other progenitor cell types, such as selected mononuclear cells, mesenchymal stem cells, or cardiac stem cells. Of note, we have recently shown a reduced migration capacity of aged BM-MNC when compared with those obtained from young healthy subjects.[32] Similarly, BM-MNC obtained from patients with diabetes mellitus or heart failure exhibit a reduced regenerative capacity.[33] Thus, it is likely that BM-MNC obtained from patients of advanced age with LV dysfunction as in most trials may be functionally deficient and hence unable to improve LVEF as has been shown in young rodent models without cardiovascular risk factors.

In conclusion, the Swiss-AMI trial in patients with STEMI and LV dysfunction intracoronary infusion of BM-MNC either 5 to 7 days or 3 to 4 weeks after successful reperfusion therapy by primary PCI did not show any improvement in LVEF at 12 months. The clinical value of BM-MNC remains unclear and, thus, the results of larger outcome studies, such as the ongoing BAMI trial (NCT 01569178), aiming to demonstrate a beneficial effect of BM-MNC on 2-year survival, must be awaited.

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Disclosures

None.

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Novelty and Significance

What Is Known?

- Earlier studies reported promising benefits in left ventricular remodeling and function using bone marrow–derived mononuclear cell treatment after acute myocardial infarction.
- The results of more recent trials using similar cell preparation techniques are challenging the initial findings. Furthermore, the durability of any positive effect has not been sufficiently demonstrated.

What New Information Does This Article Contribute?

- The long-term results of the Swiss-AMI trial are strikingly in line with most recent findings in the field lacking of any positive effect on cardiac function, remodeling, or adverse events neither 4 months nor 12 months after myocardial infarction.
- Mortality, even after large myocardial infarction, is surprisingly low, which is gratifying for patients but may have important impacts for the design of future trials.

Little more than 10 years ago, bone marrow–derived progenitor cells were first injected in the infarct-related artery several days after myocardial infarction and initial results seemed to be promising in terms of improvement of left ventricular function. The aim of the presented trial was to confirm the favorable effect on remodeling for the cell therapy groups and to address the ideal timing for treatment. We randomly assigned the 200 included patients to an open-labeled control and 2 therapy arms. Bone marrow–derived progenitor cells were, thus, injected early (5–7 days) or late (3–4 weeks) after myocardial infarction. Like at short term, also after 12 months, we could not find any benefit from progenitor cell therapy on left ventricular function or major cardiac adverse events. Only the trends of N-terminal pro-brain natriuretic peptide over time may be more favorable for the cell therapy groups than for control groups. Overall, the mortality after myocardial infarction was rather low (2.25%) without significant difference between groups. This may have important impact on the design of future trials. Concluding, intracoronary infusion of bone marrow–derived progenitor cells either early or late after large acute myocardial infarction did not improve left ventricular function at 12 months follow-up, compared with an open-labeled, randomized control group.

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The effect of bone marrow derived mononuclear cell treatment, early or late after acute myocardial infarction: Twelve months CMR and long-term clinical results

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SUPPLEMENTAL MATERIAL

Additional information concerning Methods

Justification of the inclusion of baseline LVEF in models:

Although the treatment arms were randomized, baseline LVEF was not completely equal in all three groups (Online Table I). Also, it has been shown in the past that baseline LVEF may play an important role in the interpretation of the results in studies with cell-based therapies after AMI. Especially in the Repair AMI trial [1], patients with a lower baseline LVEF derived the most benefit from cell therapy. Furthermore in the Regent trial [2], only for patients with the most depressed LVEF there was a trend in favor of cell therapy. Therefore, the conclusion has been drawn, that the difference between control and both therapy groups for baseline LVEF, although not completely significant when applying the Bonferroni correction, could not be ignored. Thus, baseline LVEF was included as covariate in all regression analyses, in order to perform adjustment for baseline LVEF.
All other confounders collected at baseline (age, gender, history of CAD, diabetes) and included in the model were chosen both due to clinical relevance and some imbalance between treatment arms observed.

Additional information concerning missingness and multiple imputation

Reason for dropping out from the study is illustrated in the flowchart of Figure 1. Already for the main publication [3], we had many discussions about drop out rate already during review process of the primary endpoint. We concluded that drop out rate was highest in the late therapy group, where patients left the hospital where primary PCI was performed and had to come back for treatment (either from home or from rehabilitation unit). This incommodity may have led to the higher drop out rate in this group. We then tried to look at clinical parameters of those patients dropping out and compared them with the remaining patients. This was not easy and contained further potential bias as some patients withdrew only partial informed consent (i.e. refused repetitive CMR analysis) whereas others withdraw complete consent including data storage or further clinical follow-up data assessment.
Taking into account all three study-groups, drop out patients were significantly older than those who maintained in the study (65years vs. 56 years). They had a trend to have higher CK max values but did not differ in terms of gender, baseline LVEF or baseline NT-proBNP.
Looking at the same values (gender, age, baseline CK max, baseline NT-pro-BNP, baseline LVEF) as per group, we did not see a difference between

drop-out and study-compliant patients for the control and for the late therapy group. However, drop-out patients in the early therapy group were older and had higher CK-max values.

We also performed a more detailed analysis of reasons for missing data:

We classified data not missing at random (Death, ICD implantation) and missing at random (MAR) which should be true for complete withdrawal from all follow-up protocols, isolated withdrawal for specific follow up protocols (no CMR for claustrophobia; refused CMR or blood sample) and for technical reasons (CMR not readable / interpretable; missing blood sample for oblivion). Most of the missing data were due to reasons concerning the clinical situation, therefore classified as MAR. Missing data classified as non-MAR, were few, i.e. death (n = 4) and ICD implantation (n = 5).

For these reasons we performed multiple imputation using chained equation, which fills in missing values in multiple variables iteratively. The Stata 14 *mi suite* was used. Fifty datasets were generated after setting a seed for reproducibility. The largest fraction of missing information (FMI) was used to confirm that the number of imputed data sets was adequate (thumb rule M=100*FMI). We imputed the LVEF, LVEDV, LVESV, scar size, NT-proBNP and their changes at 12 months. We used the following independent variables for imputation: age, body mass index, gender, hypertension, dislipidemia, Diabetes, Smoking (active/previous), Familiar history of coronary artery disease, 1 / 2 / 3 vessel disease, treatment arm, time from pain to revascularization, TIMI flow before PCI, TIMI flow after PCI, Use of Glycoprotein IIb/IIIa inhibitors, maximal creatin kinase values, initial heart failure, initial ventricular fibrillation. The imputed data were then described with the mean and standard error (SE) or the median and SE obtained via quantile regression. The same regression models as in the complete case analysis were fitted (while accounting for imputation).

Additional information for results

Comparison of the two treatment arms with controls, pooled:

Changes over time were compared between control and one pooled treatment arm (Online Figure I). The results were not different from the main analysis, with no significant interaction term, thus no claim of treatment effect could be made in this case either.

Comparison of treatment effect after multiple imputation:

Results of treatment comparisons after multiple imputation are shown in the Table 1B. They appear similar to the complete analysis, both in the data description and the comparison of time profile (p for interaction non significant in all cases); details of the regression models are reported in the Online Table IIIB.

References

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Online figure legends

Online Figure I:
Descriptive statistic (columns) of the mean absolute change in left ventricular ejection fraction (LVEF - %), left ventricular end-diastolic volume (LVEDV - ml), left ventricular end-systolic volume (LVESV – ml) and NT-proBNP (pg/ml) from baseline to 12 months for control and the combined bone marrow–derived mononuclear cell treatment group (“early + late”).

Online Tables

Online Table I: Baseline characteristics of the included patients

	Control (n = 67)	Early (n = 65)	Late (n = 63)	p-value
Age – years (median; IQR)	56 (14.5)	55 (15)	62 (15)	0.70 * 0.06 ‡
BMI - kg/m2 (median; IQR)	26.7 (4.4)	27.0 (6.1)	27.0 (4.4)	0.92 * 0.89 ‡
Male gender - %	83.6	86.2	82.5	0.18 * 1.00 ‡
Hypertension - %	43.3	49.2	38.7	0.60 * 0.72 ‡
Hyperlipidemia - %	44.8	40.0	41.9	0.60 * 0.86 ‡
Diabetes - %	17.9	7.7	9.7	0.12 * 0.21 ‡
Smoking (active/previous) - %	62.7	67.7	40.3	0.60 * 0.01 ‡
Familiar history of CAD - %	35.8	26.1	24.2	0.26 * 0.18 ‡
1 / 2 / 3 vessel disease %	64/21/15	54/32/14	57/27/16	0.34 * 0.73 ‡
Previous PCI before AMI - %	3.0	3.1	1.6	1.00 * 1.00 ‡
Infarct treatment				

Primary PCI – %	94.0	98.5	100.0	0.37 * 0.12 ‡
Concomitant PCI other than infarct related artery – %	18.2	12.3	11.1	0.47 * 0.32 ‡
Infarct vessel LAD/LCX/RCA -%	89/3/8	95/2/3	92/3/5	0.51 * 0.89 ‡
Pain to revascularization time (h)	4.5 (5)	4.8 (5.4)	4.0 (4.8)	0.57 * 0.53 ‡
Stent diameter (mm)	3.5 (0.5)	3.0 (0.5)	3.5 (0.5)	0.73 * 0.89 ‡
Drug eluting stent – %	71.6	80.0	81.0	0.31 * 0.23 ‡
TIMI flow before PCI	0 (0)	0 (0)	0 (0)	0.31 * 0.87 ‡
TIMI flow after PCI	3 (0)	3 (0)	3 (0)	0.94 * 0.81 ‡
Use of Glycoprotein IIb/IIIa inhibitors - %	64.2	70.8	58.7	0.27 * 0.32 ‡
Bivalirudin - %	7.5	7.7	19.1	0.61 * 0.04 ‡
Maximal creatin kinase - U/l (median; IQR)	3671 (3685)	4314 (3561)	3436 (3813)	0.22 * 0.78 ‡
nt-pro BNP - ng/l (median; IQR)	1103 (1848)	1450 (1442)	1581 (1912)	0.15 * 0.10 ‡
Intra aortic balloon pump / other assist device - %	16.4	15.6	22.6	1.00 * 0.18 ‡
CMR characteristics of the LV				

LVEF – % (median;IQR)	39.6 (11.2)	34.6 (16.1)	35.6 (11.2)	0.07 * 0.03 ‡
LVEDV – ml (median;IQR)	154 (44)	153 (49)	149 (47)	0.89 * 0.96 ‡
LVESV – ml (median;IQR)	94 (35)	94 (41)	97 (38)	0.54 * 0.41 ‡
Scar mass – g (median;IQR)	39.1 (37.2)	37.7 (32.1)	33.9 (24.2)	0.94 * 0.21 ‡
Myocardial scar – % (median;IQR)	28.3 (16.3)	28.1 (16.2)	26.6 (15.9)	0.78 * 0.53 ‡
MVO – g (median;IQR)	0.27 (1.55)	1.08 (3.00)	0.64 (2.49)	0.11 * 0.51 ‡

Modified from Sürder et al. *Circulation 2013* [1]
IQR: interquartile range; BMI: body mass index; CAD: Coronary artery disease; PCI: Percutaneous coronary intervention; AMI: acute myocardial infarction; LAD: left anterior descending coronary artery; LCX: left circumflex artery; RCA right coronary artery; TIMI: Thrombolysis in Myocardial Infarction
*: p value control vs. early
‡: p value control vs. late

Online Table II: Characteristics of BM-MNC and cell treatment

	Early	Late	p-value (between group difference)
Cell characteristics			
BM aspiration volume (ml)	65 (15)	70 (15)	0.30
Total MNC (10^6 cells)	159.7 (125.8)	139.5 (120.5)	0.18
Viability - %	93.6 (5.55)	93.33 (6.60)	0.98
% CD 34+ cells	1.02 (0.72)	1.31 (0.97)	0.01 #
Total CD 34+ cells (10^6 cells)	1.6 (1.69)	1.45 (2.43)	0.68
% CD 34+/133+ cells	0.81 (0.78)	0.87 (0.97)	0.34
Total CD 34+/133+ cells (10^6 cells)	0.96 (1.46)	0.92 (2.06)	0.77
% Invasion	33 (18) *	26.5 (16.5) **	0.18
Invasion index	50.88 (24.38)*	45.64 (22.10) **	0.21
Timing of BM-MNC treatment			
Days after AMI	6 (2)	24 (7)	NA

Taken from Sürder et al. *Circulation 2013* [1]

BM: bone marrow; MNC: mononuclear cells; CD 34+ cells: mononuclear cells expressing the CD34 molecule; CD 34+/133+ cells: mononuclear cells co-expressing the CD34 and CD133 protein; % invasion: percentage of mononuclear cells showing invasion capacity

#: estimated Wilcoxon effect -0.31, 95% CI -0.56,-0-07); *: n = 29; **: n = 30

Online Table III - Multivariable Models (p value for interaction is in bold & red)

A Complete Case Analysis

LVEF	Coef.	Robust Std. Err.	t	P>t	[95% CI]	
TREATMENT						
early	-0.53	0.33	-1.571	0.118	-1.19	0.13
late	-0.62	0.37	-1.691	0.093	-1.34	0.10
MONTHS						
4	-0.53	1.15	-0.465	0.642	-2.79	1.73
12	-1.89	1.36	-1.390	0.166	-4.56	0.79
TREATMENT#MONTHS				**0.640**		
early# 4	2.28	1.60	1.426	0.156	-0.87	5.42
early#12	1.10	1.99	0.552	0.582	-2.83	5.04
late# 4	1.40	1.59	0.885	0.377	-1.73	4.53
late#12	1.23	2.08	0.592	0.555	-2.88	5.35
LVEFbaseline	0.86	0.04	20.892	0.000	0.78	0.94
age	-0.01	0.04	-0.189	0.850	-0.08	0.07
gender	1.54	1.38	1.112	0.268	-1.19	4.26
DM	-0.18	1.17	-0.155	0.877	-2.49	2.13
CAD	0.04	0.59	0.073	0.942	-1.12	1.21
_cons	4.33	2.82	1.537	0.126	-1.23	9.89
Model	*F (13, 174)*	*61.11*	*p<0.001*	*R-squared*	*0.56*	

B Multiple imputation

LVEF	Coef.	Robust Std. Err.	t	P>t	[95% CI]	
TREATMENT						
early	-0.47	0.60	-0.790	0.432	-1.65	0.71
late	-0.51	0.64	-0.796	0.428	-1.78	0.76
MONTHS						
4	-0.54	1.19	-0.450	0.653	-2.89	1.82
12	-1.43	1.30	-1.098	0.274	-4.00	1.14
TREATMENT#MONTHS				**0.688**		
early# 4	2.07	1.65	1.251	0.213	-1.20	5.34
early#12	0.83	1.89	0.439	0.661	-2.90	4.55
late# 4	1.27	1.66	0.766	0.445	-2.00	4.54
late#12	1.51	1.90	0.795	0.428	-2.25	5.28
LVEFbaseline	0.86	0.04	21.255	0.000	0.78	0.94
age	-0.01	0.04	-0.187	0.852	-0.08	0.07
gender	1.31	1.29	1.012	0.313	-1.25	3.86
DM	0.35	1.17	0.295	0.768	-1.97	2.66
CAD	-0.10	0.57	-0.171	0.864	-1.22	1.03
_cons	4.46	2.95	1.509	0.134	-1.38	10.30
Model	*F (13, 183)*	*42.02*	*p<0.001*			

LVEDV	Coef.	Robust Std. Err.	t	P>t	[95% CI]	
TREATMENT						
early	-2.13	6.67	-0.319	0.750	-15.30	11.05
late	2.31	6.18	0.373	0.709	-9.89	14.50

LVEDV	Coef.	Robust Std. Err.	t	P>t	[95% CI]	
TREATMENT						
early	-3.25	6.85	-0.474	0.636	-16.77	10.28
late	0.85	6.55	0.130	0.897	-12.10	13.80

MONTHS						
4	27.88	5.04	5.528	0.000	17.92	37.83
12	18.04	7.14	2.528	0.012	3.95	32.13
TREATMENT#MONTHS				**0.063**		
early# 4	-0.61	6.52	-0.093	0.926	-13.47	12.25
early#12	6.84	9.43	0.725	0.470	-11.78	25.45
late# 4	-17.92	7.34	-2.442	0.016	-32.41	-3.44
late#12	-8.55	9.69	-0.882	0.379	-27.68	10.58
LVEFbaseline	-1.84	0.28	-6.631	0.000	-2.38	-1.29
age	-0.71	0.25	-2.801	0.006	-1.20	-0.21
gender	-27.29	7.64	-3.572	0.000	-42.37	-12.21
DM	9.12	13.34	0.684	0.495	-17.20	35.45
CAD	-0.82	4.44	-0.186	0.853	-9.58	7.93
_cons	296.16	20.17	14.683	0.000	256.35	335.97
Model	*F (13, 174)*	*10.75*	*p<0.001*	*R-squared*	*0.25*	

MONTHS						
4	25.67	5.71	4.496	0.000	14.38	36.95
12	16.56	7.16	2.312	0.022	2.41	30.72
TREATMENT#MONTHS				**0.292**		
early# 4	-1.12	7.58	-0.148	0.883	-16.11	13.87
early#12	4.27	9.81	0.435	0.664	-15.11	23.65
late# 4	-16.40	8.39	-1.956	0.053	-32.99	0.19
late#12	-10.22	10.32	-0.990	0.324	-30.64	10.20
LVEFbaseline	-1.84	0.26	-7.199	0.000	-2.34	-1.33
age	-0.78	0.25	-3.089	0.002	-1.28	-0.28
gender	-26.26	7.30	-3.596	0.000	-40.69	-11.83
DM	6.36	11.43	0.557	0.578	-16.20	28.93
CAD	-0.27	4.13	-0.064	0.949	-8.42	7.89
_cons	300.73	19.97	15.063	0.000	261.29	340.17
Model	*F (13, 183)*	*8.29*	*p<0.001*			

LVESV	**Coef.**	**Robust Std. Err.**	**t**	**P>t**	**[95% CI]**	
TREATMENT						
early	-2.12	4.44	-0.478	0.633	-10.89	6.64
late	0.28	4.03	0.069	0.945	-7.68	8.23
MONTHS						
4	19.34	4.23	4.570	0.000	10.98	27.69
12	16.83	6.06	2.778	0.006	4.87	28.79
TREATMENT#MONTHS				**0.199**		
early# 4	-2.74	5.78	-0.474	0.636	-14.15	8.67
early#12	3.24	8.34	0.389	0.698	-13.22	19.71
late# 4	-12.97	6.18	-2.099	0.037	-25.17	-0.78
late#12	-7.66	8.24	-0.929	0.354	-23.93	8.61

LVESV	**Coef.**	**Robust Std. Err.**	**t**	**P>t**	**[95% CI]**	
TREATMENT						
early	-2.67	4.75	-0.562	0.575	-12.05	6.71
late	-0.86	4.59	-0.187	0.852	-9.92	8.21
MONTHS						
4	17.86	4.78	3.738	0.000	8.41	27.30
12	15.02	5.97	2.518	0.013	3.23	26.80
TREATMENT#MONTHS				**0.490**		
early# 4	-3.05	6.57	-0.464	0.643	-16.04	9.94
early#12	1.79	8.33	0.215	0.830	-14.67	18.26
late# 4	-11.87	7.03	-1.690	0.093	-25.77	2.03
late#12	-9.08	8.59	-1.057	0.292	-26.06	7.91

LVEFbaseline	-2.55	0.22	-11.645	0.000	-2.99	-2.12
age	-0.45	0.20	-2.199	0.029	-0.85	-0.05
gender	-18.33	6.60	-2.776	0.006	-31.37	-5.30
DM	4.66	10.35	0.450	0.653	-15.77	25.09
CAD	-0.19	3.65	-0.051	0.959	-7.39	7.01
_cons	241.07	16.04	15.028	0.000	209.41	272.73
Model	*F (13, 174)*	*15.19*	*p<0.001*	*R-squared*	*0.36*	

LVEFbaseline	-2.56	0.20	-12.634	0.000	-2.96	-2.16
age	-0.50	0.21	-2.428	0.016	-0.92	-0.09
gender	-17.34	6.27	-2.766	0.006	-29.73	-4.95
DM	1.99	8.93	0.222	0.824	-15.65	19.62
CAD	0.47	3.41	0.139	0.890	-6.25	7.19
_cons	244.37	16.09	15.191	0.000	212.59	276.15
Model	*F (13, 183)*	*14.13*	*p<0.001*			

Infarct size (g)	**Coef.**	**Robust Std. Err.**	**t**	**P>t**	**[95% CI]**	
TREATMENT						
early	-3.86	4.36	-0.884	0.378	-12.47	4.76
late	-9.18	4.42	-2.079	0.039	-17.90	-0.47
MONTHS						
4	-16.39	2.97	-5.522	0.000	-22.25	-10.53
12	-23.91	3.49	-6.846	0.000	-30.81	-17.02
TREATMENT#MONTHS				**0.181**		
early# 4	0.74	3.88	0.192	0.848	-6.92	8.40
early#12	4.65	4.39	1.058	0.291	-4.02	13.32
late# 4	2.34	4.24	0.551	0.582	-6.02	10.69
late#12	8.70	5.03	1.730	0.085	-1.22	18.62
LVEFbaseline	-0.83	0.10	-8.404	0.000	-1.02	-0.63
age	0.10	0.09	1.141	0.255	-0.07	0.28
gender	-8.96	2.25	-3.981	0.000	-13.40	-4.52
DM	1.46	2.70	0.541	0.589	-3.86	6.78
CAD	-1.83	1.18	-1.556	0.122	-4.16	0.49
_cons	85.35	8.16	10.458	0.000	69.24	101.46
Model	*F (13, 174)*	*16.50*	*p<0.001*	*R-squared*	*0.36*	

Infarct size (g)	**Coef.**	**Robust Std. Err.**	**t**	**P>t**	**[95% CI]**	
TREATMENT						
early	-2.01	4.35	-0.462	0.645	-10.60	6.58
late	-8.42	4.23	-1.989	0.048	-16.77	-0.07
MONTHS						
4	-14.69	3.00	-4.900	0.000	-20.61	-8.76
12	-20.74	3.28	-6.331	0.000	-27.21	-14.27
TREATMENT#MONTHS				**0.702**		
early# 4	-0.40	4.12	-0.098	0.922	-8.54	7.73
early#12	1.88	4.47	0.420	0.675	-6.96	10.71
late# 4	1.89	4.27	0.442	0.659	-6.55	10.33
late#12	6.00	4.75	1.262	0.209	-3.39	15.39
LVEFbaseline	-0.86	0.10	-8.307	0.000	-1.06	-0.65
age	0.11	0.10	1.106	0.270	-0.09	0.30
gender	-8.78	2.62	-3.347	0.001	-13.97	-3.58
DM	1.25	2.85	0.440	0.661	-4.37	6.88
CAD	-1.72	1.29	-1.333	0.185	-4.26	0.83
_cons	84.45	9.01	9.370	0.000	66.65	102.25
Model	*F (13, 183)*	*13.20*	*p<0.001*			

ln-proBNP	Coef.	Robust Std. Err.	t	P>t	[95% CI]	
TREATMENT						
early	0.13	0.15	0.831	0.407	-0.17	0.42
late	0.11	0.17	0.674	0.501	-0.22	0.44
MONTHS						
4	-1.01	0.14	-7.123	0.000	-1.29	-0.73
12	-1.32	0.18	-7.470	0.000	-1.66	-0.97
TREATMENT#MONTHS				**0.404**		
early# 4	-0.14	0.17	-0.858	0.392	-0.48	0.19
early#12	-0.25	0.20	-1.219	0.225	-0.65	0.15
late# 4	-0.12	0.19	-0.607	0.544	-0.50	0.26
late#12	-0.33	0.23	-1.460	0.146	-0.78	0.12
LVEFbaseline	-0.04	0.00	-7.760	0.000	-0.05	-0.03
age	0.03	0.00	5.584	0.000	0.02	0.04
gender	0.31	0.14	2.163	0.032	0.03	0.59
DM	0.02	0.17	0.108	0.914	-0.33	0.36
CAD	-0.05	0.08	-0.604	0.547	-0.20	0.11
_cons	6.78	0.41	16.656	0.000	5.98	7.59
Model	*F (13, 172)*	*52.89*	*p<0.001*	*R-squared*	*0.49*	

ln-proBNP	Coef.	Robust Std. Err.	t	P>t	[95% CI]	
TREATMENT						
early	0.20	0.15	1.336	0.183	-0.10	0.50
late	0.06	0.17	0.331	0.741	-0.28	0.39
MONTHS						
4	-0.89	0.14	-6.207	0.000	-1.17	-0.61
12	-1.19	0.17	-6.959	0.000	-1.53	-0.86
TREATMENT#MONTHS				**0.736**		
early# 4	-0.23	0.18	-1.244	0.215	-0.59	0.13
early#12	-0.31	0.21	-1.472	0.143	-0.73	0.11
late# 4	-0.12	0.21	-0.561	0.576	-0.54	0.30
late#12	-0.23	0.24	-0.962	0.337	-0.71	0.25
LVEFbaseline	-0.04	0.01	-7.965	0.000	-0.05	-0.03
age	0.03	0.00	5.759	0.000	0.02	0.04
gender	0.29	0.14	2.041	0.043	0.01	0.57
DM	-0.02	0.17	-0.090	0.929	-0.35	0.32
CAD	-0.04	0.07	-0.542	0.589	-0.19	0.11
_cons	6.71	0.42	15.884	0.000	5.87	7.54
Model	*F (13, 183)*	*34.61*	*p<0.001*			

Online Table III reports the entire statistical results obtained with STATA: Modeling of LVEF, LVEDV, LVESV, MI mass and ln-proBNP (log-transformed NT-proBNP) as dependent variables and treatment (TREATMENT), months of follow-up (MONTHS) and their interaction (TREATMENT#MONTHS) as independent variables. The purpose of the model is to verify whether profiles over time differ between treatment arms by testing this interaction. The p-value for interaction is reported in red and bold in the TREATMENT#MONTHS line.
Coef.: Regression coefficient; Robust Std. Err.: Huber-White robust standard error; t: t statistic to that tests whether coefficients are = 0; P>t: Corresponding p-value; [95% Conf. Interval]: 95% confidence interval of the regression coefficient.
Model F statistic and p-value, together with the model explained variation (R squared) are reported below each model report.
Both the analysis on raw data (A) and the analysis on multiple imputation (B) are shown.

Online Figure I: